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Original article

Development of green tea infused chocolate yoghurt and evaluation of its nutritive value and storage stability

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Summary. The yoghurt industry is presently booming with the introduction of new tart flavors in the row while green tea is emerging as a proven health-beneficial drink in the market. An amalgamation of the two brings in a healthy, tasty and filling platter in today's food souk. Besides, addition of a hint of chocolate will help to enrich the flavor of the product by masking the bitterness of green tea. A blend of all these will surely be satisfying both the nutrition and taste quotient, as desired by today's health-conscious and indulgence-seeking consumers in the growing markets. With this view, green tea infused chocolate flavored yoghurt was developed from double toned milk with infusion of green tea and addition of chocolate syrup at the rate of 2% (w/v) and 9% (v/v) respectively. Yoghurt was procured from local market and used as starter culture. The innoculum level of 2% (w/v) yielded a product having good setting properties with no syneresis which was preferred most by the sensory panelists. After setting the yoghurt, honey was added to obtain a product with smooth texture, semisolid consistency and sweet taste and aroma. A storage study of the final product was also carried out against plain yoghurt in refrigerated condition (7±1°C) whereby its sensory, physico-chemical and microbiological quality were monitored to approximately determine its shelf life. The shelf life of the formulated yoghurt was found to be 21 days under refrigerated conditions as compared to 15 days for plain yoghurt under same condition.

Key words: green tea, yoghurt, formulation, syneresis, sensory quality, shelf life

1. Introduction

Yoghurt is a cultured milk product prepared by controlled fermentation of milk by *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (1, 2) and also by *Lactobacillus helvetius* and *Lactobacillus delbrueckii* spp. (3).Yoghurt with probiotic cultures helps to improve certain gastrointestinal conditions, including glactose intolerance (4-6);constipation, diarrhea and dysentery;colon cancer (7); inflammatory bowel disease; *Helicobacterpylori* infection(8) etc. The benefits are thought to be due to changes in the microflora of the gut, the time food takes to go through the bowel and enhancement of the body's immune system. Recently, it has been an increasing trend to fortify yoghurt with functional ingredients to modify its appearance, functional property, nutritional quality and texture. Several attempts had been made for successful fortification of yoghurt with whey powder (9, 10), soy products (11), iron (12), calcium (13, 14), PUFA (15), fruit extracts (16), garlic (17), green tea extracts (18-20) etc. The cholesterol increasing property of milk fat in yoghurt attracted imaginative food technologists to develop reduced fat yoghurt with improved organoleptic, physico-chemical, rheological, textural and nutritional properties. Addition of whey protein

concentrate (21) andstarch lipid composite (22)were indicative approach towards the use of fat replacer in yoghurt. Inulin, the polysaccharide obtained from the roots of *Chicoriumintybus* was successfully incorporated into yoghurt to provide a fat like mouthfeel and texture in low fat yoghurt along with having a prebiotic effect (23-26). Another study suggested lowering of postprandial appetite ratings but not energy intakes in young healthy females after feeding yoghurt breakfast added with inulin (27). Also it was reported that fortification of yoghurt with protein and guar gum may prevent weight gain by controlling short term appetite (28).

Green tea, the second basic ingredient of the product, has increasingly grabbed the attention of consumers since the last decade during which, its manifold health-promoting properties have come into the limelight. Green Tea (*Camellia sinensis*, family Theaceae) is brewed from the un-oxidized, dried leaves of the plant and hence has the maximum amount of polyphenols (30%-45% of green tea solids by weight) intact in it (29). The major polyphenolic constituents present in green tea are epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG) and epigallocatechin-3-gallate (EGCG) and gallocatechin (GC) (30). The green tea catechins are powerful antioxidants those traps reactive oxygen species and remove endogenously generated superoxide, peroxyl and hydroxyl free radicals, which are mainly responsible for chronic degenerative diseases (31, 32). The order of effectiveness as radical scavengers is ECG-EGCG-EGC-EC-GC (33). Green tea has also been reported to beneficially affect the consumers with anti-stress (34), anticancer (35, 36), neuroprotective effects (37), and helps in reduction of atherosclerosis, cardiovascular diseases, obesity problems, diabetes, pulmonary ailments, osteoporosis and diseases of the kidneys and liver (38, 39).They have also been claimed to possess antibacterial and antiviral effects (40-42). The low pH value, of approximately 4.2, makes tea compatible with many food products in term of acidity (43). The presence of green tea alters the coagulation of milk protein and subsequently modifies the textural and rheological properties of yoghurt (19).

The primary reason for adding chocolate and honey is to enhance the flavor and taste of the product as green tea does not boast of a rich flavor itself. Several research works indicate that cocoa or dark chocolate has one of the richest sources of flavanol antioxidants with 610 mg total catechins/kg of fresh edible weight (44). This polyphenols include theobromine, phenethylamine, epicatechin, procyanidins and caffeine (45). Chocolates also contain minerals, specifically potassium, magnesium, copper and iron (46). Chocolate phenols may positively affect arteries by reducing blockages; reducing intestinal inflammation, resisting oxidative stress and inhibiting lipid oxidation etc (47).

Therefore, the main objective of the present work was to formulate green tea infused chocolate yoghurt stirred with honey in order to amalgamate probiotic effect of yoghurt with antioxidant activity of green tea along with a delicious note of chocolate that would satiate both the healthy and taste quotient of consumers.

2. Material and Methods

2.1 Materials

Double toned milk and sugar was procured from the local market. AMUL yoghurt was used as starter culture. Green tea bags used for infusion, was manufactured by Twinings India Ltd. AMUL Skim milk powder, Dabur honey and Hershey's chocolate syrup were also purchased from local market. All the chemicals were purchased from HIMEDIA® and are of analytical grade.

2.2 Preparation of yoghurt

A previously developed method with little modification was used to prepare green tea infused yoghurt (18). The flow diagram was given in Figure 1. Double toned milk was scalded upto about 80-85°C for 10-5 minutes with 1% (w/v)addition of skim milk powder. During the heating process of milk, green tea bags (2% w/v) were dipped and allowed to stand for 20 minutes for extraction of tea polyphenols. Thereafter, 9% (v/v) chocolate syrup was added to the scalded milk and was cooled up to 40°C.The milk was then inoculated with 2% (w/v) starter culture, mixed properly and incubated at 25°C for 14-16 hours until desired consistency has reached. Thereafter, 3% (w/v) honey was added, stirred to a homogeneous and smooth consistency and stored

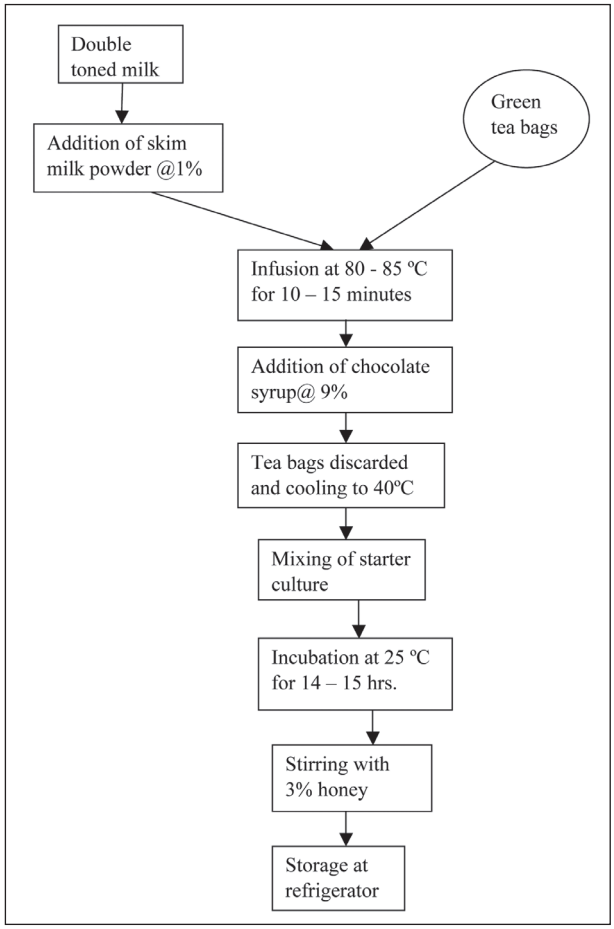

Figure 1. Flow Diagram for manufacturing of green tea infused chocolate yoghurt with honey

in a refrigerator (5±2˚C). A similar set of experimental design was carried out with the addition of 3% (w/v) sugar instead of honey. Also, a control was prepared by inoculating only boiled milk with the same culture under similar conditions of the experiment.

2.3 Sensory analysis

Formulated yoghurt of various combinations was judged by a semi-trained sensory panel comprising of Departmental faculty and staff members. Five sensory characteristics of the product i.e. color, flavor, mouthfeel, taste and overall acceptability were evaluated using 9-point hedonic scale (9=like very much; 1=dislike very much) (49). Each sample weighing 5g under chilled condition (4˚C) were presented to panelists in identical plastic cups labeled with random numbers and in random order. The panels recognized the yogurt only by codes. Sensory panelists were instructed to rinse their mouth by drinking plain water after assessment of each sample. All assessments were done in individual booths of a sensory laboratory under white light illumination (color temperature: 6500 K)

2.4 Physico-chemical analysis

The pH of the yoghurt samples was determined using the digital pH meter (Model No. 5633, ECIL, India). Titratable acidity and total solids content was determined according to the Association of Official Analytical Chemists (AOAC, 2000) method (50). For determination of titratable acidity 10 g sample was diluted with 10 ml distilled water and titrated against 0.1(N) NaOH solution using phenolphthalein indicators. Total solid was determined by Oven drying methods by heating the sample at 100±2˚C for 4 hours. Total carbohydrate was determined by Anthrone method. Total fat, protein and ash contents were determined according to the AOAC method (50). The total polyphenolic compounds of green tea extract as well as the green tea infused chocolate yoghurt samples were analyzed. The total polyphenol content (TPC) of sample was determined by spectrophotometer (UV-1800 ENG 240V, Shimadzu), using gallic acid as standard, according to the Folin-Ciocalteu method as described by the International Organization for Standardization (ISO) 14502-1 (51). The sample was centrifuged (Remi BL-24, VCAC-049, Mumbai, India) at 10000 g for 15 minutes at 5˚C and the supernatant was filtered through 0.45m filter paper. 0.1 ml of the diluted supernatant was transferred in duplicate to separate tubes containing 1.5 ml of 1:1 dilution of Folin-Ciocalteu's reagent in water. Then, 4.0 ml of sodium carbonate solution (20% w/v) was added followed by volume make up of 10 ml with distilled water. The tubes were then allowed to stand at room temperature for 30 min and absorbance was taken at 738 nm against a blank. The TPC was expressed as gallic acid equivalents (GAE) in g/100 g material. The concentration of polyphenols in samples was derived from a standard curve of gallic acid ranging from 10 to 50 µg/ml (Pearson's correlation coefficient: r^2=0.9996) and results were expressed as micrograms of gallic acid equivalents (GAE) per milliliter of sample.

Syneresis was carried out using a standard method (52). A cup of yoghurt was weighed accurately and the container was then positioned at an angle of 45° for 2 hours at 4°C. The whey accumulated was removed from the yoghurt surface by using a syringe and the cups were then re-weighed. Syneresis was reported in terms of the percentage of whey lost using the following formula: Syneresis (%) = (whey lost/sample weight) x 100%.

2.5 Microbiological analysis

Microbiological quality of the beverage samples were periodically analyzed during storage taking 10 ml representative samples and aseptically mixed with 90 ml distilled water and homogenized by shaking. Subsequent decimal dilutions were prepared with the same diluents and in all cases duplicate-counting plates were prepared of appropriate dilutions. The total viable counts and yeast and mold counts in the samples were determined according to APHA (53) using nutrient agar and potato dextrose agar respectively.

2.6 Statistical analysis

All experiments were performed in triplicate and results were expressed as mean ± standard deviation. Differences between group means were estimated using a one way ANOVA followed by Duncan's multiple range test (54) using SPSS Statistic 17.0 (IBM, India), for sensory analysis of different yoghurt formulations. The Duncan's multiple range tests was conducted in between the sample for particular sensory attributes. Result were considered statistically significant when $p<0.05$.

3. Results and Discussion

3.1 Sensory analysis for optimization of yoghurt

In the present study, the inoculum level (w/v) was optimized varying the level of starter culture in the range of 0.5%, 1%, 1.5%, 2% and 2.5%. On the basis of sensory evaluation and titratable acidity values as obtained for the samples, 2% (w/v) inoculum level was selected. Titratable acidity value for 0.5%, 1%, 1.5%, and 2.5% inoculated yoghurt samples were not complying with the legally permitted requirements of country specific regulatory guideline standard, Food Safety and Standard Aurhority of India (FSSAI, 2011) which should be between 0.85-1.2 % in terms of lactic acid. In addition to this, yoghurt samples with lower than 2% (w/v) inoculum size, showed poor setting of curd, excessive visible syneresis and therefore scored poorly on the sensory characteristics of mouthful and overall acceptability. So, 2% (w/v) inoculums size was chosen for development of plain yoghurt and green tea infused chocolate yoghurt. The green tea infused chocolate yoghurt was then stirred with honey and sugar separately to increase the consumer acceptability. All the samples were evaluated on the basis of sensory parameters (color, flavor, mouthfeel, taste and overall acceptability) by the panelists.

In the present investigation, the sensory quality of developed products were compared statistically using Duncan's multiple range tests in term of colour, flavor, mouthfeel, taste and overall acceptability. Table 1 represents the average value of different sensory parameters for different yoghurt samples. It was observed that the sensory attributes namely color, flavor, mouthfeel, taste, and overall acceptability has increased due to incorporation of formulation ingredients like green tea, chocolate, sugar, and honey in yoghurt which significantly ($p<0.05$) differ with respect to plain yoghurt. It was illustrated from Table 1 that sensory value of color for green tea infused chocolate yoghurt stirred with honey was significantly higher than other samples. The sensory analysis in term of flavor showed that plain yoghurt had significantly ($p<0.05$) lower sensory value than the other developed product. The highest flavor value was shown by green tea infused chocolate yoghurt stirred with honey. Almost similar type of result was observed for the mouthfeel and taste. It may be due to the incorporation of honey which increases acceptability of the product by imparting some desirable color, flavor and taste. Moreover, the overall acceptability of the developed product was also varied significantly and the highest acceptability was observed for green tea infused chocolate yoghurt stirred with honey. Based on sensory analysis green tea infused chocolate yoghurt stirred with honey was most acceptable and henceforth it was used as sample for further studies.

3.2 Analysis of nutritional quality

The nutritional quality of the green tea chocolate yoghurt stirred with honey referred as sample, plain

Table 1. Sensory analysis of different yoghurt samples

Sample	Colour	Flavour	Mouthfeel	Taste	Overall Acceptability
Plain yoghurt	7.5±0.24[a]	7.26±0.26[a]	7.04±0.28 [a]	7.14±0.31[a]	7.20±0.26[a]
Green tea infused chocolate yoghurt	8.06±0.24[b]	7.69±0.27[b]	7.30±0.26 [b]	6.64±0.29[b]	7.35±0.20[a]
Green tea infused chocolate yoghurt with sugar	8.10±0.20[b]	7.95±0.32[c]	7.94±0.33[c]	7.65±0.35[c]	7.88±0.33[b]
Green tea infused chocolate yoghurt with honey	8.36±0.32[c]	8.32±0.24[d]	8.52±0.21[d]	8.12±0.25[d]	8.37±0.26[c]

**Average of 3 trials judged by 7 panelists*
**Means with different letters in the same row indicate that there is significant difference between samples (p≤0.05) from Duncan's multiple range tests.*

yoghurt referred as control and green tea extracts was represented in table 2. It reveals that the total solids content of sample is higher as compared to control which may be due to incorporation of chocolate syrup and honey in the sample and the proportion of addition of ingredients were mentioned in the formula. The fat, protein and total carbohydrate content of sample is slightly higher than the control. The total phenolic content in the pure green tea extract prepared by brewing 2 gm sample in 100 ml water and the developed sample were found to be 256.31±12.34 µgm/ ml and 5.21±0.14 µgm/ ml respectively measured in terms of gallic acid equivalent.

3.3 Changes in yoghurt during refrigerated storage

The optimized yoghurt sample which is prepared by fermentation of double toned milk infused with green tea and chocolate syrup and stirred with honey was stored in the refrigerator (7±2°C) in packaged form. During storage the sensory, physico-chemical and microbiological changes was evaluated at regular interval to approximately determine its storage stability. The values were compared with plain yoghurt as control.

Table 1 represents the changes in sensory properties of control and sample during refrigerated storage. The scores reveal that after 21 days of storage, sensory attributes of the sample yoghurt decreased considerably while that for control decreased significantly. The reduced rate of fermentation by lactic acid bacteria in sample yoghurt was due to the presence of antimicrobial factors present in infused green tea (55) and honey. This impacted the minimal changes in organoleptic properties of the samples during storage. Microbial hydrolysis of yoghurt components mainly lactose, protein and fat during storage plays pivotal role in maintaining sensory attributes and ultimately determines the shelf life of the product. From the results it was clear that the plain yoghurt has spoiled on 18th day of storage while the green tea infused chocolate yoghurt

Table 2. Nutritional analysis of samples

Parameters	Control	Sample	Green tea extract
Total solids (%)	12.28±0.34	18.04±0.22	-
Fat (%)	3.65±0.35	3.96±0.21	-
Protein (%)	4.53±0.30	4.88±0.24	-
Total carbohydrate (%)	5.29±0.18	6.21±0.33	-
Ash (%)	0.91±0.01	0.93±0.01	-
Total polyphenol (in terms of gallic acid equivalent) µgm/ ml	-	5.21±0.14	256.31±12.35

**Average of three trials*

Table 3. Changes in sensory properties during storage

Days of storage	Flavour		Colour& appearance		Mouthfeel		Overall acceptability	
	Control	Sample	Control	Sample	Control	Sample	Control	Sample
0	8.1	8.32	8.5	8.75	8.43	8.4	8.32	8.5
3	7.75	8.25	8.31	8.65	8.27	8.25	8.14	8.36
6	7.38	7.91	8.16	8.52	8.06	8.07	8.0	8.25
9	7.06	7.75	7.91	8.25	7.9	8.0	7.72	8.03
12	6.75	7.38	7.75	8.01	7.76	7.75	7.35	7.8
15	6.4	7.14	7.31	7.65	7.25	7.6	7.04	7.5
18	5.92	6.86	7.15	7.38	6.92	7.25	6.25	7.07
21	5.25	6.43	7.0	7.14	6.5	6.9	5.75	6.65

Table 4. Changes in microbiological quality during storage

Days of storage	Total bacterial count (cfu/ml)		Yeast and mold count (cfu/ml)	
	Control	Sample	Control	Sample
0	15×10^4	11×10^4	10	9
3	76×10^4	52×10^4	13	13
6	132×10^4	81×10^4	24	18
9	21×10^5	97×10^4	33	23
12	45×10^5	142×10^4	43	31
15	97×10^5	22×10^5	58	38
18	39×10^6	38×10^5	76	49
21	78×10^6	64×10^5	94	62
24	37×10^7	12×10^6	118	68

was acceptable up to 21 days. This observation can be explained in the light of the fact that green tea extract possesses antimicrobial properties due to the presence of polyphenolic catechins (55). So, the presence of green tea in the sample has extended the shelf-life of the product. It has also been observed that the complete spoilage of green tea infused chocolate yoghurt took place on 24th day of storage.

During refrigerated storage the changes in physicochemical properties of the yoghurt samples are represented in figure 2. The results clearly state that the titratable acidity of the control kept on increasing sharply and pH, hence, decreasing sharply from the day of preparation up till the 21st day. The same trend is noticed in case of the sample which is actually chocolate flavored yoghurt with honey as the sweetening agent. The overall increase in the titratable acidity or decrease in pH is less probably due to the antimicrobial properties of green tea and honey. The same trend is noticed for total solids as well as for syneresis during the storage period.

The changes in microbiological quality of the yoghurt samples during storage are represented in the table 2. The total bacterial count for both the control and the sample steadily increased from an initial value of 15×10^4 to 37×10^7 cfu/ ml on 24th day of storage for control and 11×10^4-2×10^6 cfu/ml for sample. An increase in acidity corresponding to reduction in poten-

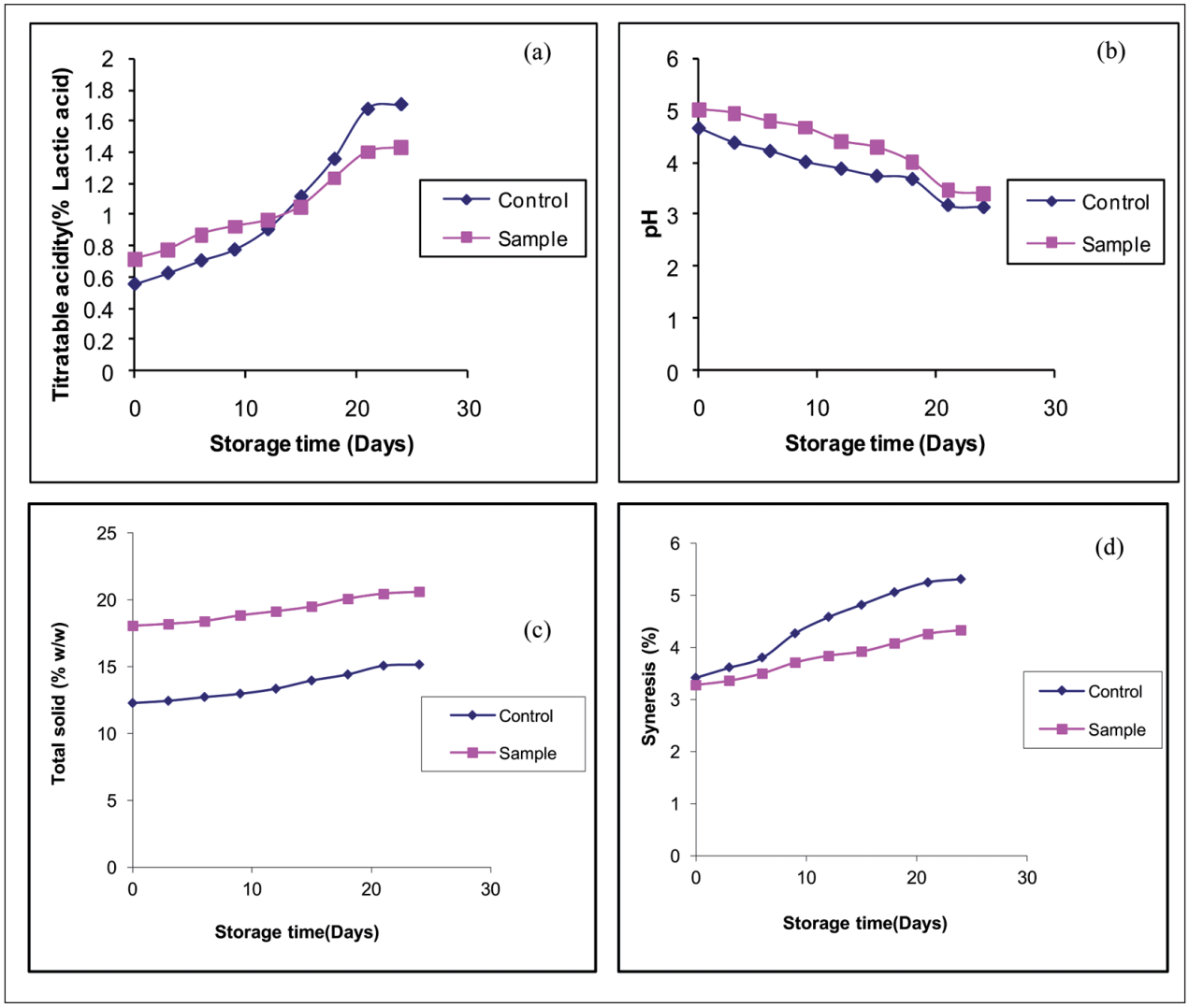

Figure 2. Changes in Physico-chemical properties during storage

tial oxygen during fermentation process may provide suitable state for growth of yeasts and molds. The yeast and mold counts also increase from 10 to 118 and 9 to 68 cfu/ ml for control and the formulated yoghurt samples respectively.

Conclusion

Healthy foods with active free radical scavengers are very popular worldwide nowadays. Keeping in mind the growing trend of the consumers' acceptance towards healthy and functional foods, an attempt has been taken to formulate green tea infused chocolate yoghurt stirred with honey in order to amalgamate probiotic effect of yoghurt with antioxidant activity of green tea. Sensory assessment is an integral part of new food product development. Without appropriate sensory analysis, there is risk of market failure because comparison and choice are fundamental to consumers. Sensory evaluation comprises a set of techniques for accurate measurement of human responses to foods and minimizes the potentially biasing effects of brand identity and other information influences on

the consumer perception. The results obtained from this experiment indicated that chocolate yoghurt prepared with green tea infusion were quite satisfactory. The product could be a viable functional food where chocolate acts as a flavoring agent and honey as the sweetening agent. Green tea did not adversely affect the growth of the bacteria as indicated in the microbiological study. The chocolate coupled with the honey helped to overcome the bitter flavor of the green tea. The product had good consumer acceptability as indicated by the sensory evaluation. The future success of this type of product in food industry depends on successful scale up operations, specific food safety and quality measures, flexibility of food processors towards developing new products in relation to changing face of consumer demand and dynamism of segmented markets in different demography.

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